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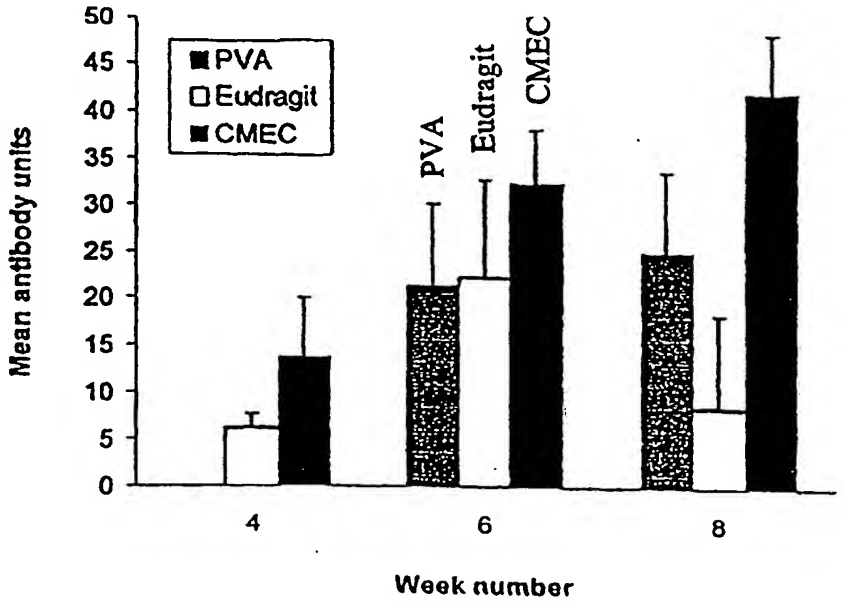

(57) Abstract

A microparticulate composition comprising a biodegradable synthetic polymer, a proteinaceous antigen and an enteric polymer, wherein the enteric polymer forms a coating layer on the surface of the particle is described.

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Microparticulate Composition

The present invention relates to a microparticulate composition and more particularly to a microparticulate drug delivery composition in which the drug is a proteinaceous antigen. The compositions of the invention may exhibit enhanced mucosal delivery.

It is known that advantages can be obtained by delivering therapeutic materials such as drugs, diagnostic agents and antigens to specific sites in the body. Various methods and systems have been proposed (see the review by Pettit and Gombotz in Trends in Biotechnology, page 343, 1998).

Microparticulate carriers in the form of microspheres and microcapsules can be used for the delivery of therapeutic materials into the blood stream, into body tissues or into the body cavities and lumens of the nose, gastrointestinal tract, vaginal cavity, etc. Such microparticulate systems are familiar to those skilled in the art (see for example the book by Davis et al. Microspheres and Drug Therapy, Elsevier, Holland, 1984).

Microparticles can be produced using a range of biodegradable and biocompatible polymers. These polymers can provide particles with different physicochemical characteristics, e.g. size, and different degradation rates as well as different levels of loading of the therapeutic agent. In the field of antigen and drug delivery, polylactide polymers and polylactide-co-glycolide polymers have been popular as materials from which microparticles can be prepared.

The delivery of microparticles containing therapeutic agents to the gastrointestinal tract of vertebrates such as fish and mammals can be

advantageous. It has been shown that such particles can be taken up by certain cells that line the gastrointestinal tract, such as the epithelial cells (enterocytes) and specialised cells called M-cells (microfold cells) located in the Peyer's patches. The cells of the colon wall, such as colonocytes and lymphoid cells, also represent suitable targets. Similar types of specialized cells are present in the nasal cavity.

The encapsulation of antigens in microparticles for use as oral vaccines has been described in the prior art. A significant proportion of the antigen may be entrapped inside the particle and therefore is not exposed to the external environment in the gastrointestinal tract. However, a further significant proportion, e.g. greater than 60 %, of the antigen may be attached to the surface of the particle. Some of the surface adsorbed material may be released quickly after administration (the so-called burst effect), but a proportion of the surface material can be tightly bound to the particle and is believed to be a critical determinant in the resultant immune response.

When a microparticle carrying an antigenic material is administered to the gastrointestinal tract of a vertebrate, the material incorporated inside the polymer matrix should be protected satisfactorily by that matrix. In contrast, the surface exposed antigenic material can be degraded or modified unfavourably by the effect of endogenous pH and enzymes. Consequently, the vaccine system will be less efficacious.

The oral administration of an active agent to the lymphoid tissue of the small intestine (Peyer's patch) using microcapsules formed from a biodegradable and biocompatible synthetic polymer, such as polylactide-co-glycolide, is described in EP-A-266119. The use of an enteric polymer to coat such particles is not described.

The enteric coating of formulations containing whole microorganisms has also been described in the prior art. For example, an early description of enteric coated particles in oral vaccine delivery involved the encapsulation of Escherichia coli heat labile enterotoxin in so-called microspheres (3 mm in diameter) prepared from starch and cellulose with hydroxypropylmethylcellulose phthalate as the enteric coating polymer (Klipstein et al. Infect. Immun. 39, 1000 (1983)). Oral administration of this formulation induced serum and intestinal antibody responses comparable to those induced following oral delivery of the antigen alone after a dose of the gastric inhibitor cimetidine. There was no suggestion that microparticles less than 1000 microns, made from synthetic polymers could be coated with an enteric layer.

Cellulose acetate phthalate has also been used to coat microspheres of 1-3 mm in size containing a virus (Maharaj et al. J. Pharm. Sci. 73, 39, 1984). The same polymer has also been used to produce microspheres with entrapped bacteria (Lin et al. J. Microencaps. 8, 317, 1991). These different formulations were designed to protect the antigen against degradation in gastric fluid and facilitate its subsequent release in the intestine. There was no suggestion that proteinaceous antigens could be entrapped in microparticles less than 1000 μm and the resulting microparticles enterically coated.

An oral vaccine comprising a live recombinant adenovirus in an enteric coated dosage form is described in GB-A-2166349. No mention is made of microparticulate polymeric carriers.

Bender et al. (J. Virol. 70, 6418 (1996)) has suggested that a replication-deficient, orally administered enteric coated vaccina virus vectored

vaccine might safely protect against influenza. Similarly Bergmann et al. (Int. Arch. Allergy Appl. Immunol. 80, 107 (1986)) administered an enteric coated inactivated influenza vaccine to 5 volunteers via the oral route. Neither of these systems comprised biodegradable microparticles made from synthetic polymers.

US-5,676,950 describes a recombinant vaccine or pox virus for oral administration where an enteric coating can be used so that the virus is released only when it reaches the small intestine. There is no description of biodegradable synthetic polymeric microparticles.

Particulate carriers having a solid core comprising a polysaccharide and a proteinaceous material and an organometallic polymer bound to the core as a protective coating are described in WO-95/31187. There is no description of biodegradable synthetic polymeric microparticles.

Oral compositions of sensitive proteinaceous agents, such as an immunological agent or vaccine, have been disclosed in US-5,032,405. The patent discloses a particulate diluent uniformly coated with an alkaline soluble polymeric coat which will dissolve at a specific pH. The polymer coat comprises at least one partially esterified methacrylic acid. The particulate diluent comprised maltose and optionally a further material such as an inorganic salt. No mention is made of a proteinaceous antigen adsorbed on the surface of biodegradable synthetic polymeric microparticles such as those formed from polylactide or polylactide-co-glycolide.

Microspheres with a core layer containing an immunogen and an enteric coating which protects and retains shape at room temperature have been described in WO-98/07443. The enteric coating is soluble in the digestive

tract and has the property of maintaining sphere structure at room temperature. The microspheres were prepared from gelatin by extruding an immunogen suspension fluid from the central tube and an aqueous solution of the enteric substance from the outer tube of a concentric multi-tube nozzle into a solution to solidify the drops. Microspheres prepared from synthetic biodegradable polymers were not described.

WO-92/00096 describes an oral vaccine composition that can be formulated as enteric dosage forms in the form of microspheres, biodegradable microcapsules or liposomes. Enteric coatings are not described.

Oral pig vaccines as enteric coated microparticles having a globular shape and critical maximum diameter are disclosed in DE 2343570. The particles have a diameter of preferably less than 1.5 mm and are coated with cellulose acetate phthalate. The core is a solid carrier such as barium sulphate. Synthetic polymer carriers are not described.

Gelatin spheres coated with an enteric film for oral administration of immunogen are described in JP-5294845. Polymeric microparticles produced from synthetic polymers were not described.

US-5,591,433 describes the microencapsulation of a protein with an aqueous solution of an enteric polymer. The protein, which can be an immunogen, is not attached to or incorporated in a polymeric microparticle. Indeed, the objective in US-5,591,433 is to allow the release of the protein into solution in the intestine to avoid degradation of the protein in the stomach.

Microparticulate oral drug delivery compositions comprising biodegradable polymeric microparticles prepared from synthetic polymers, a proteinaceous antigen encapsulated by and surface adsorbed on the microparticles and a protective coating of an enteric polymer over the surface of the microparticles have not been previously described. Furthermore, the preparation of such microparticles by a water-in-oil-in-water double emulsion process in which the enteric polymer is used as the stabilising agent has not been previously described.

We have now developed a biodegradable microparticulate drug delivery composition which is adapted for oral administration in which the microparticles carry a surface layer of an enteric polymer that protects surface adsorbed antigen from degradation or modification in the gastrointestinal tract and particularly the stomach of an animal. The protective coating of the enteric polymer can lead to an improved immune response when the microparticles are administered orally to an animal.

By biodegradable we mean a material that can degrade upon administration to a living organism such as a mammal or fish. The degradation may be through the non-specific cleavage of chemical bonds, such as hydrolysis of an ester, or through an enzyme catalyzed process. The degradation results in the synthetic polymer decreasing in molecular weight so that the polymeric microparticle eventually dissolves and is no longer resident in the body as an intact particle.

For the case of biodegradable microparticles in the form of microspheres or microcapsules, these can degrade over a period of days, weeks, or months depending on their chemical composition and molecular weight. Degradation can be via a process of surface or bulk erosion or a combination of these processes.

An enteric (or gastro-resistant) polymer is defined as a material that does not dissolve in the stomach of an animal at acidic pH values, but when the polymer transits to the intestines, where the pH is higher than that of the stomach, the polymer will start to dissolve. The threshold pH for such dissolution to occur will depend on the chemical nature of the polymer. Typically enteric polymers contain weak acid groups that can ionize at pH values above their pKa values and start to dissolve. A review on enteric polymers by Healy can be found in Ch 7, Drug Delivery to the Gastrointestinal Tract, Hardy, Davis, Wilson (eds), Ellis Horwood, Chichester, 1989.

According to a first aspect of the present invention, there is provided a microparticulate composition comprising a biodegradable synthetic polymer, a proteinaceous antigen and an enteric polymer, wherein the enteric polymer forms a coating layer on the surface of the microparticles.

The microparticulate composition of the invention may be used for delivering drugs. The composition comprises polymeric microparticles which are made from a biodegradable synthetic polymer and which are loaded with the proteinaceous antigen. The enteric polymer forms a coating or layer on the surface of the microparticles.

It will be appreciated that the enteric polymer will not necessarily coat the entire outer surface of the microparticles. Typically, from 40 to 100% of the outer surface of the microparticles will be covered by the enteric polymer. Preferably at least 60% of the surface will be covered and most preferably at least 80% of the surface will be covered.

By a microparticulate composition we mean a composition which is comprised of microspheres and/or microcapsules. By a microparticle, we mean a particle that is less than 1000 μm in diameter comprising a matrix of the biodegradable synthetic polymer which carries the proteinaceous antigen. We prefer a particle diameter in the range 0.1 to 20 μm, more preferably in the range 0.5 to 10 μm and most preferably in the range 1.0 to 5.0 μm. The antigen may be dispersed within the microsphere, on the surface of the microsphere or more typically will be divided between these two locations. Such surface adsorbed antigen can be important to the correct presentation of the antigen to the cells of the immune system.

By a microcapsule, we mean a hollow or voided particle which comprises one or more hollows or voids which are surrounded by a matrix formed from the biodegradable synthetic polymer. The proteinaceous antigen is located in the hollow or void(s) of the capsule and on its surface. In one particular embodiment, the microcapsule comprises a centrally located hollow which contains a proportion of the proteinaceous antigen and a surrounding shell or casing which is formed from the biodegradable synthetic polymer.

Whether the microparticle is a microsphere or a microcapsule, the enteric polymer forms a coating on the outer surface of the particle and protects surface adsorbed antigen from degradation or modification.

Microparticles for the improved delivery of antigens can be made from synthetic biodegradable polymers using methods known in the art such as emulsification, phase separation and spray drying (see the article by Kissel et al. Chp. 10. in Antigen delivery systems, Eds., Gander et al. Harwood Academic Publishers, Netherlands, 1997).

In the spray drying process, the material used to form the body of the microparticles is dissolved in a suitable solvent (usually water) and the solution spray dried by passing it through an atomisation nozzle into a heated chamber. The solvent evaporates to leave microparticles.

Preferred emulsification methods are the water-in-oil-in-water and the water-in-oil-in-oil double emulsification methods.

The water-in-oil-in-water double emulsification method involves the preparation of a water-in-oil-in-water emulsion. The antigen is dissolved in water or an aqueous solution containing a buffer and/or other formulation components such as sugars, cyclodextrins, etc. The aqueous solution of the antigen is then emulsified in an immiscible oil phase comprising an organic solvent in which the biodegradable synthetic polymer is dissolved, to produce a water-in-oil emulsion (w/o). A stabilising agent can be used in the preparation of this initial w/o emulsion. The choice of organic solvent will be dictated by the properties of the biodegradable polymer. Suitable solvents include, inter alia, dichloromethane, ethylacetate, ethyl formate and chloroform. The solubility product concept may be used to select an appropriate polymer/solvent combination. The resultant water-in-oil emulsion is then re-emulsified into an aqueous phase to produce a double water-in-oil-in water emulsion (w/o/w). The second (external) aqueous phase contains an agent that will stabilise the double emulsion and the microparticles which are formed such as polyvinylalcohol (PVA). In a preferred embodiment, the enteric polymer is used as the stabilising agent in the second aqueous phase (see *infra*). The organic solvent is then removed by evaporation or extraction resulting in the formation of rigid microparticles where the contents of the internal aqueous phase which include the antigen are entrapped to a lesser or greater extent inside the biodegradable polymer.

The water-in-oil-in-oil method is described in PCT/GB95/01426 (Yeh et al.). In this process, an aqueous solution of the material to be encapsulated (e.g. protein) is emulsified with a first organic solvent (e.g. dichloromethane). This water-in-oil emulsion is then mixed with a solution of biodegradable polymer (e.g. poly-L-lactide), dissolved in the same (e.g. dichloromethane) or a second organic solvent. Finally, this mixture is emulsified with a third organic solvent (e.g. methanol), which is miscible with the first and second organic solvents, but is not a solvent for the polymer, to form a water-in-oil-in-oil emulsion. The emulsion is stirred until the dispersed phase solvent (e.g. dichloromethane) is extracted. The microparticles thus formed are washed several times in water and freeze dried.

Of the above techniques for making microparticles, the water-in-oil-in-oil and especially the water-in-oil-in-water methods are preferred.

The therapeutic antigen is incorporated in or onto the microparticle to a varying degree of efficiency. This can be from less than 0.01% w/w to greater than 40% w/w loading on the total weight of the microparticle depending on the nature of the polymeric material used for the microparticles as well as the properties of the therapeutic antigen and the processing method. The antigen can be loaded onto the microparticles after they have been prepared and isolated providing that this is done before the microparticles are coated with the enteric polymer. Generally, however, the antigen is incorporated during the manufacturing process used to make the microparticles and will tend to collect inside the microparticles as well as being adsorbed on the outer surface of these particles.

The enteric polymer which coats the surface of the microparticles may be applied to already formed microparticles, e.g. prepared as described above, using coating techniques known in the art such as spraying, and dipping.

Thus, in accordance with a second aspect of the present invention there is provided a process for preparing a microparticulate composition comprising polymeric microparticles formed from a biodegradable synthetic polymer, a proteinaceous antigen carried by the microparticles and a coating of an enteric polymer on the surface of the microparticles, which process comprises forming the polymeric microparticles carrying the antigen and coating the surface of the so formed microparticles with an enteric polymer.

However, we have discovered that it is possible to produce the microparticles of the present invention by a water-in-oil-in-water emulsion technique in which the enteric polymer is used as a stabilising agent during the preparation of the microparticles rather than the more usual stabilising agents such as polyvinyl alcohol. The enteric polymer can preferentially locate at the surface of the microparticles during the manufacturing process in much the same way as conventional stabilising agents and thereby encourages the formation of discreet, non-aggregated microparticles.

With this technique, microparticles carrying a surface layer of the enteric polymer are prepared in a single step process so that there is no need to carry out a discrete coating step to apply the enteric layer.

Thus, in accordance with a third aspect of the present invention there is provided a process for preparing a microparticulate composition

comprising polymeric microparticles formed from a biodegradable synthetic polymer, a proteinaceous antigen carried by the microparticles and a coating of an enteric polymer on the surface of the microparticles, which process comprises forming the polymeric microparticles in the presence of the antigen and the enteric polymer. In a preferred embodiment, the process is an emulsification process, particularly a water-in-oil-in-water emulsification process, in which the enteric polymer acts as a stabiliser for the microparticles which are formed in the process.

Suitable biodegradable synthetic polymers for use in the present invention include, but are not limited to, polylactide, polylactide-co-glycolide, polycaprolactone, polyhydroxyalkanoates, polyothoesters, polyanhydrides, polyphosphazenes, polyalkylcyanoacrylates, polymalic acids, polyacrylamide, polylactide-PEG, polyethyleneglycol copolymers and polycarbonates. These polymers can be processed to produce rigid microparticles.

Polylactide-co-glycolide is a preferred polymer for the microparticle. The molar ratio of lactide to glycolide can be from 10 to 90%. 50:50 and 75:25 mixtures on a molar basis of lactide to glycolide are preferred. The molecular weight of the polylactide-co-glycolide polymer can be from 2 kD to 200 kD. A molecular weight from 10 to 50 kD is preferred.

Polylactide is another preferred biodegradable polymer for the microparticles. The molecular weight of this polymer can be from 1 kD to 400 kD. A material with a molecular weight in the range 2 to 10 kD is preferred.

Suitable enteric polymers include, *inter alia*, cellulose acetate trimelletate, hydroxypropylmethylcellulosephthalate, polyvinylacetatephthalate,

cellulose acetate phthalate, shellac, methacrylic acid copolymers such as Eudragit L-100-55 which is an anionic copolymer based on methacrylic acid and ethyl acrylate and is described in the United States Pharmacopeia/National Formulary as a methacrylic acid copolymer, type C. Carboxymethylethyl cellulose (CMEC) is a preferred material. Commercially available CMEC has a mean molecular weight of 49 kD. The content of carboxymethyl and ethoxyl groups in the polymer can be in the range of from 8.9 to 14.9 and 32 to 43% w/w%, respectively.

A proteinaceous antigen is one that is obtained from the surface or core of a virus or is the surface or internal material of a bacteria or parasite. The protein can be a glycoprotein such as GP120 known for the HIV virus. Examples of proteinaceous materials include the nuclear proteins of influenza, surface proteins of influenza and pertussis, fimbrial proteins of E. coli toxoid and toxins. The antigen can be prepared from a microorganism or through a process of genetic engineering where a construct (fusion protein) can be grown in a bacterial or mammalian cell etc. Such constructs can include the antigen together with a material that can improve the performance of the vaccine such as a cytokine (interleukin) or immunostimmulatory peptide. The proteinaceous antigen can be a component of the diet that may give rise to allergy such as ovalbumin or proteins from shell fish or peanuts.

By controlling the thickness of the enteric coating it will be possible to deliver the surface attached antigen undamaged to the distal small intestine (ileal region) or to the various parts of the large intestine.

The composition can be delivered bucally, orally, rectally, nasally, conjunctivally or via the genitourinary tract or via any appropriate method to a mucosal surface of a vertebrate. Oral delivery is preferred.

The microparticles of the present invention will be particularly useful for the oral immunisation of animals (for example by addition to the feed) or for fish (administration to aquaculture) and to children who find difficulty in swallowing solid dosage forms such as tablets and capsules.

The present invention is now illustrated but not limited with reference to the following examples.

Example 1 Preparation of Microspheres with Enteric Polymers and Entrapped Bioactive Agents

Method:

An aqueous solution of ovalbumin (OVA) in distilled water (2 ml, 30 mg/ml) was emulsified with 10 ml of a 6% solution of polylactide-co-glycolide (50:50 polylactide:polyglycolide, 34,000 D molecular weight; Boehringer Ingleheim, Ingleheim, Germany) in dichloromethane using a Silverson homogeniser for 2 minutes at high speed (12,000 rpm) to produce a primary water in oil emulsion. This water-in-oil (w/o) emulsion was then emulsified at high speed with a 10% solution of an enteric polymer as stabiliser to produce a water-in-oil-in-water (w/o/w) emulsion. Either carboxymethylethyl cellulose (CMEC, Freund, Japan) or Eudragit L-100-55 (Rohm Pharma, Germany) were used as the enteric polymers and different concentrations of these polymers were used and were buffered to a final pH of 6. The w/o/w emulsion was stirred for approximately 18 hours at room temperature and pressure to allow solvent evaporation and microsphere formation. The microspheres were isolated by centrifugation, washed and freeze-dried. The microspheres were

examined by scanning electron microscopy for surface morphology and size analysed by laser diffractometry (Malvern - Mastersizer).

Results:

The microparticles stabilised using the enteric polymers displayed a spherical shape and smooth surface and were non-porous. The sizes of the microparticles are as shown below as d(50%) μm, d(10%) μm and d(90%) μm which are the sizes obtained by laser diffractometry as percentage undersize.

		Particle Size		
Stabiliser	Stabiliser (%) w/v	d(50%) (μm)	d(10%) (μm)	d(90%) (μm)
Eudragit	2.5	1.31	0.43	4.08
Eudragit	4	0.96	0.52	2.39
Eudragit	6	0.81	0.44	4.01
CMEC	4	0.54	0.26	1.28
CMEC	6	0.56	0.26	1.07
CMEC	8	0.40	0.19	1.97

Example 2 Entrapment of Bioactive Materials in Microspheres with Enteric Polymers

Methods:

Microspheres stabilised with enteric polymers and containing OVA were prepared as described in Example 1. The OVA was extracted from the

microspheres by one of two means: i) microparticles (3-4 mg) were shaken overnight with 1 ml of 0.1 M sodium hydroxide solution; ii) microparticles (10 mg) were suspended in 0.25 ml of 5% aqueous sodium dodecyl sulphate solution and shaken for 1 hour, then 1 ml of 50:50 dichloromethane:acetone was added and the sample stirred overnight to evaporate the organic solvents. These samples were then analysed for OVA content using a BCA protein microassay and also by SDS-PAGE assay (Laemmli, Nature 227, 600-605, 1970). The amount of OVA present was determined against a series of OVA standards in suitable buffers (in triplicate).

Results:

The amount of OVA entrapped in each of the formulations was as below:

Stabiliser	Stabiliser (%) w/v	Encapsulation Efficiency[a] (%)	OVA Load (%) w/w by BCA	OVA Load (%) (w/w) by SDS-PAGE
Eudragit	2.5	38.3	3.5	3.1
Eudragit	4	62.7	5.7	4.7
Eudragit	6	19.0	1.7	2.0
CMEC	4	48.1	4.4	4.4
CMEC	6	30.0	2.7	1.6
CMEC	8	34.4	3.1	2.4

(a) The encapsulation efficiency is defined as the quantity of material (OVA) encapsulated with respect to the amount in the original aqueous solution used to prepare the initial water in oil emulsion.

Example 3 Preparation of Microspheres Loaded with a Model Antigen (Ovalbumin)

Microspheres similar to those described in the prior art were prepared as described in Example 1 except that the aqueous phase used as the external phase in the water in oil in water emulsion contained 10% w/v polyvinyl alcohol (PVA) (87-89% hydrolyzed, average molecular weight 13 kD - 23 kD as obtained from Aldrich, Gillingham, UK) as stabiliser instead of an enteric polymer. The resulting microspheres did not, therefore, have an enteric coating and are not part of the present invention, but are used as controls in subsequent examples.

The encapsulation efficiency was 54%. The measured particle diameters were d(50%) 0.49 μm, d(10%) 0.25 μm and d(90%) 0.96 μm, respectively. The OVA loading as measured by BCA assay and SDS PAGE, respectively, were 4.92% w/w and 3.43% w/w.

Example 4 Surface Localisation and Release of Bioactive Materials in Microspheres with Enteric Polymers

Methods:

The release of OVA from the microparticles was evaluated. OVA-loaded microparticles were incubated for 1 hour in acid medium, (0.5 ml, 0.7% v/v HCl + 0.2% w/v NaCl aqueous solution, pH 1.2) at 37°C to simulate the stomach. The microparticles were then isolated by centrifugation and re-suspended in pH 7.4 phosphate buffered saline (PBS) at 37°C to simulate the intestines. At intervals over a 7 day period, samples of PBS were removed and assayed for OVA content. Fresh medium was added to each sample of microparticle suspension to replace the volume removed.

The acid and PBS samples were analysed for OVA content by a BCA assay.

The level of surface-located OVA was measured by incubating the microparticles in 0.5 ml PBS, pH 7.4 containing 30 μg pepsin or in simulated gastric fluid (pH 1.2, 3.2 mg/ml pepsin). The level of OVA was assayed via BCA and its structural integrity via SDS-PAGE, plus Western Blotting where appropriate.

Results:

The release studies showed that OVA was not released from microparticles stabilised with enteric polymer (CMEC) when incubated for 1 h at pH 1.2. In contrast, microparticles prepared using a non-enteric stabiliser (polyvinyl alcohol, PVA) released 13.5% of the total OVA content at pH 1.2. When the medium was changed to pH 7.4 PBS, only an additional 5% OVA was released after 2 days from the PVA-stabilised microparticles. In contrast, more than 15% of the OVA load was released from the CMEC-stabilised microparticles within the same time period at pH 7.4.

Treatment of CMEC-stabilised microparticles with pepsin resulted in the loss of some OVA (measured by BCA), but significantly less than that observed with PVA-stabilised microparticles. This demonstrated that the surface layer of enteric polymer could substantially protect an attached antigen from disadvantageous modification by the pH and enzymes present in the stomach of an animal.

Stabiliser	Stabiliser (%) w/w	Loss of microsphere-associated OVA(%) after incubation with pepsin
CMEC	4	4.0
CMEC	6	4.4
CMEC	8	13.1
PVA (Not enteric)	10	44.7

The microparticle morphology was maintained after incubation of the CMEC stabilised microspheres in pepsin and gastric media as assessed by electron microscopy.

The percentage of OVA remaining intact after treatment with simulated gastric fluid was determined by SDS-PAGE. It was found that following treatment with the simulated gastric fluid, a greater percentage of intact OVA was present in the CMEC-stabilised microspheres (33 - 61%) than in microspheres stabilised with the non-enteric polymer PVA (less than 30% intact OVA). Western Blotting showed that when using the enteric polymers as a microsphere stabiliser an increased amount of intact and antigenic OVA was associated with the microspheres (over formulations prepared without enteric polymer).

This data indicates that the stabilisation of microspheres with enteric polymers confers a significant degree of protection of antigenic agents as compared to equivalent systems prepared with a non-enteric stabilising agent.

Example 5 **Surface Localisation of Enteric Polymers on the Microspheres**

Methods:

The surface localisation of the stabilising enteric polymers was measured using two different techniques. Standards of enteric polymers, OVA and other stabilisers were appropriately made.

The surface localisation of the two enteric polymers, CMEC and Eudragit L-100-55, and polyvinyl alcohol (PVA) (control) microspheres was determined by X-ray photoelectron spectroscopy (XPS) and static secondary ion mass spectroscopy (SSIMS). XPS spectra were acquired using a VG Scientific ESCALAB Mark II instrument employing Mg Kα X-rays and electron take-off angles of 35° and 65° relative to the sample surface giving analysis depths of 3 mm and 5 mm. The X-ray gun was operated at 10 KeV and 20 mA. Survey spectra were run with a pass energy of 50 eV and high resolution. Peak areas were calculated after subtraction of linear background and spectra fitted with Gaussian peaks with 20% Lorentzian character.

SSIMS spectra were collected using a VG Ionex SIMSLAB3B instrument equipped with a differentially pumped EX05 ion gun and a 12-12M quadrapole mass spectrometer. An argon atom beam was utilised with a total dose per sample below a 10^{13} atoms/cm^2 threshold.

Results:

XPS showed that the surfaces of the microspheres were well covered with enteric polymers but the coverage was not 100% complete. SSIMS

showed that use of the two enteric polymers as stabilisers reduced the amount of surface located OVA and effectively covered what was present as compared to microspheres made with PVA.

Example 6 Improvements in Performance of Microspheres made with Enteric Polymer Stabilisers

Methods:

The *in vivo* performance of the antigenic agent OVA (0.1 mg) adsorbed and entrapped within the microspheres stabilised with enteric polymers, was assessed in an immunogenicity model in mice. Groups of 8 week old female BALB/c mice (n=8) were immunised by oral gavage on three consecutive days with 0.1 mg OVA as follows:

1. Microspheres with 10% w/v PVA as stabiliser. (Control)
2. Microspheres with 4% w/v Eudragit L-100-55 as stabiliser.
3. Microspheres with 4% w/v CMEC as stabiliser.

The microspheres were prepared as in Example 1 (for microsphere systems 2 and 3) and as in Example 3 (for microsphere system 1). Doses were administered in a volume of 0.5 ml distilled water. An identical series of booster immunisations were carried out 4 weeks later. Blood and saliva were collected by approved methods prior to immunisation, at 4 weeks following primary immunisation and at 2 and 4 weeks following boost immunisation. Serum was collected by centrifugation and stored until required. Saliva was collected according to the same schedule as above. Specific IgG and IgA anti-OVA antibodies generated in the mice were detected by a specific ELISA assay, as known to the person skilled in the art. Mean values were compared using an unpaired Students t-test

to assess statistical significance. Results were considered significant if $p < 0.05$.

Results:

The levels of specific IgG anti-OVA antibodies detected in the serum were raised after boost immunisation (see Figure 1). IgG levels are expressed in mean antibody units. The levels of specific IgG elicited to OVA associated with the CMEC formulation were significantly higher than the other formulations at week 8. The levels of specific IgA anti-OVA antibodies detected in saliva were raised after boost immunisation (Figure 2). IgA levels obtained by ELISA are expressed as optical density measurements at a wavelength of 405 nm (Titertek multiscan ELISA reader). The highest levels were detected in mice immunised with microspheres stabilised with CMEC. CMEC microspheres induced antibody levels in saliva that were significantly higher ($p<0.05$) than those elicited after immunisation with microspheres not stabilized with enteric polymers (PVA). Two weeks after boosting, anti-OVA levels were 9-fold higher with CMEC microspheres than the levels found for PVA microspheres.

Claims

1. A microparticle composition comprising a biodegradable synthetic polymer, a proteinaceous antigen and an enteric polymer, wherein the enteric polymer forms a coating layer on the surface of the particle.

2. A microparticle composition according to Claim 1, wherein the enteric polymer was used to stabilise the microparticles during the preparation thereof.

3. A composition according to Claim 1 and 2, wherein the enteric polymer is carboxymethylethylcellulose.

4. A composition according to Claim 1 and 2, wherein the enteric polymer is a methacrylic acid polymer.

5. A composition according to Claim 1 and 2, wherein the biodegradable synthetic polymer is a polylactide-co-glycolide.

6. A composition according to any one of Claims 1 to 4, wherein the biodegradable synthetic polymer is selected from the group consisting of polylactide, polycaprolactone, polyhydroxyalkanoates, polyorthoesters, polyanhydrides, polyphosphazenes, polyalkylcyanoacrylates, polymalic acids, polyacrylamide, polylactide-polyethylene glycol copolymer and polycarbonates.

7. A composition according to any one of Claims 1 to 6 which is adapted for mucosal delivery.

8. A composition according to Claim 7 which is adapted for oral delivery.

9. A composition according to Claim 1, wherein the biodegradable microparticle is made from polylactic acid, polyglycolic acid or a copolymer of these two materials (polylactide-co-glycolide).

10. A composition according to Claim 9, wherein the molar ratio of lactide:glycolide units in the copolymer ranges from 10:90 to 90:10.

11. A composition according to any one of claims 1 to 10, wherein the microparticles have a diameter less than 1000 μm.

12. A process for preparing a microparticulate composition comprising polymeric microparticles formed from a biodegradable synthetic polymer, a proteinaceous antigen carried by the microparticles and a coating of an enteric polymer on the surface of the microparticles, which process comprises forming the polymeric microparticles in the presence of the antigen and the enteric polymer.

13. A process as claimed in Claim 12, wherein the process is an emulsification process.

14. A process as claimed in Claim 13, wherein the process is a water in oil in water emulsification process in which the enteric polymer acts as a stabiliser for the microparticles which are formed in the process.

15. A process as claimed in Claim 13 which is a double or single-emulsification process in which the biodegradable polymer is dissolved in

a suitable solvent and then emulsified using an aqueous solution of the enteric polymer.

16. A process as claimed in any one of claims 12 to 15, which results in a microparticulate formulation in which the microparticles have a size range 200 nm to 1000 μm.

17. The use of a composition according to any one of Claims 1 to 11 for oral or mucosal vaccination.

18. A method of enhancing the delivery of an oral or mucosal vaccine which comprises using a microparticle composition according to Claim 1 to deliver the vaccine to an animal.

19. A microparticle composition comprising a biodegradable synthetic polymer, a proteinaceous antigen and an enteric polymer, wherein the enteric polymer forms a coating layer on the surface of the microparticles and wherein the microparticles were formed in the presence of the antigen and the enteric polymer.

20. A microparticle composition as claimed in Claim 19, wherein the microparticles were formed by an emulsification process.

21. A microparticle composition as claimed in Claim 20, wherein the microparticles were formed by a water-in-oil-in-water emulsification process in which the enteric polymer acted as a stabiliser for the microparticles which were formed in the process.

22. A microparticle composition as claimed in Claim 20, wherein the microparticles were formed by a double or single-emulsification process

in which the biodegradable polymer was dissolved in a suitable solvent and then emulsified using an aqueous solution of the enteric polymer.

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Figure 1

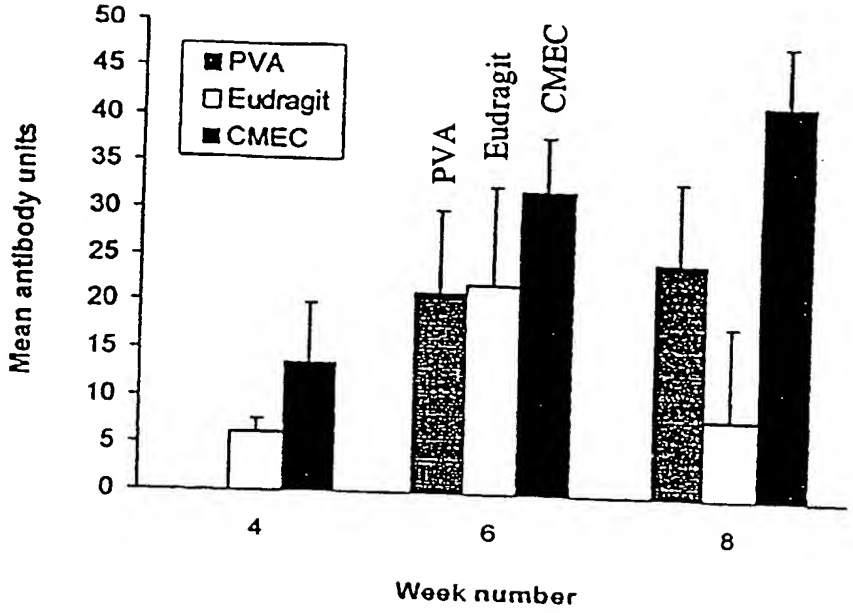
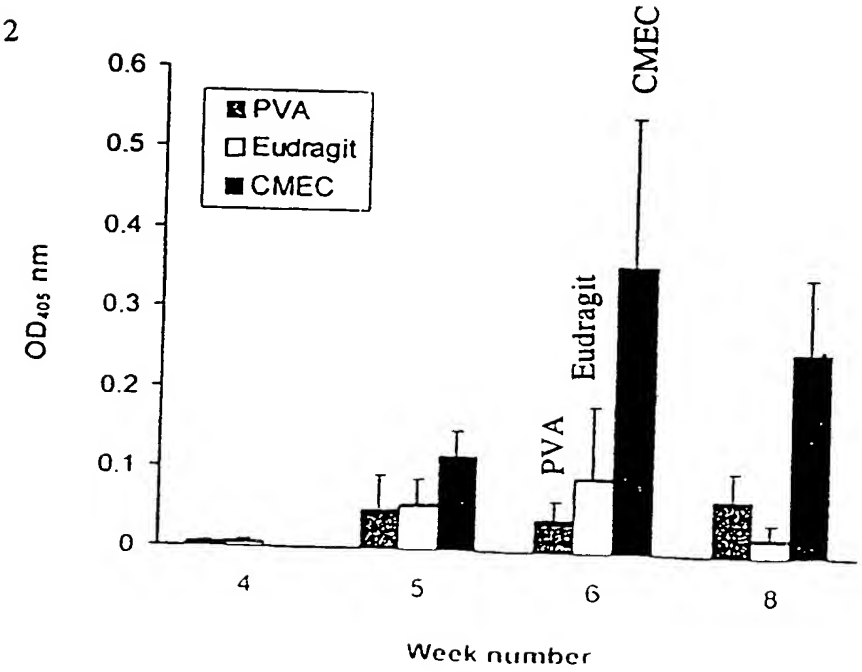


Figure 2



INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/GB 99/02775

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/16 A61K9/50 A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 603 992 A (UNIVERSITY OF CINCINNATI) 29 June 1994 (1994-06-29) claims 1-7 & US 5 591 433 A cited in the application	1-22
A	A.G.A. COMMBES ET AL.: "the control of protein release from poly (dl-lactide co-glycolide) microparticles by variation of the external aqueous phase surfactant in the water-in-oil-in water method" JOURNAL OF CONTROLLED RELEASE, vol. 52, no. 3, 31 March 1998 (1998-03-31), pages 311-320, XP004115441 Amsterdam (NL) the whole document	1-22

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 December 1999	17/12/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Benz, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/GB 99/02775

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 09956 A (CORIXA CORPORATION ET AL.) 4 March 1999 (1999-03-04) page 55 -page 56; example 1	1,2,5-22
T	Z. LU ET AL. : "A new stabilizer used in microencapsulation" JOURNAL OF MICROENCAPSULATION, vol. 16, no. 4, 1 July 1999 (1999-07-01), pages 523-529, XP000829610 London (GB) the whole document page 527; figure 4	1,2,5-22
A	WO 98 14179 A (CIMA LABS, INC.) 9 April 1998 (1998-04-09) claim 1	3

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/GB 99/ 02775

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 17 and 18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/GB 99/02775

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 603992 A	29-06-1994	AT 143803 T	15-10-1996
		AU 664222 B	09-11-1995
		AU 3104593 A	07-07-1994
		CA 2086631 A,C	23-06-1994
		DE 69305313 D	14-11-1996
		DK 603992 T	24-03-1997
		ES 2095001 T	01-02-1997
		GR 3022090 T	31-03-1997
		JP 7010771 A	13-01-1995
		NZ 245618 A	26-10-1994
		SG 52402 A	28-09-1998
		US 5609871 A	11-03-1997
		US 5629001 A	13-05-1997
		US 5591433 A	07-01-1997
		US 5783193 A	21-07-1998
WO 9909956 A	04-03-1999	AU 9125698 A	16-03-1999
WO 9814179 A	09-04-1998	AU 4664297 A	24-04-1998